Paper No. 22

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte JIANYUN DONG and RAYMOND A. FRIZZELL

Appeal No. 1997-2139
Application No. 08/114,595[1]

ON BRIEF

Before WILLIAM F. SMITH, SPIEGEL, and MILLS Administrative Patent Judges.

SPIEGEL, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 1 through 46 and 49. Claim 50, the only other claim pending in this application, has been withdrawn from further consideration under 37 CFR § 1.142(b) as not readable on the elected invention.

[1] Application for patent filed August 31, 1993.

Claims 1, 7, 23, 25-27, 30 and 49 are illustrative and read as follows.

1. An adenovirus or herpes virus vector construct comprising a recombinant insert including an expression region comprising an essential adeno-associated virus (AAV) gene, the vector expressing an essential AAV protein.[2]

7. The vector construct of claim 1, wherein the vector is a herpes simplex virus (HSV), a cytomegalovirus (CMV), a pseudorabies virus (PRV) or an Epstein-Barr Virus (EBV) vector .

23. A recombinant host cell incorporating a vector construct in accordance with claim 1.

25. The recombinant host cell of claim 23, wherein the cell further includes a recombinant AAV vector integrated into its genome.

26. The recombinant host cell of claim 25, wherein the AAV vector includes AAV ITR [i.e., inverted terminal repeat] sequences and an expression region encoding an exogenous protein.

27. The recombinant host cell of claim 26, wherein the AAV vector includes an expression region encoding a full length cystic fibrosis transmembrane conductance regulator (CFTR) protein.

30. A method of producing recombinant AAV virions, comprising the steps of:

(a) preparing a recombinant adenovirus or herpes virus which includes a vector construct comprising an essential AAV

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[2] "The terms 'essential AAV genes' and 'essential AAV protein' are intended to refer to those genes, and their encoded proteins, which are normally encoded by wild type AAV and are required for AAV replication, genome packaging and virion assembly. Naturally, when intended for use in AAV production, the adenoviral or herpes virus vector will be constructed so the inserted AAV genes complement any essential AAV genes which have been deleted from a recombinant AAV vector to allow an exogenous gene, such as a therapeutically important gene, to be inserted into the AAV vector." [Specification, para. bridging pp. 7-8.]

"The ITR sequences are the only essential cis-acting elements for an AAV vector to mediate genome packaging and integration into host cells" (specification, p. 6, ll. 31-33), while "the AAV genes (rep-lip-cap) [are] essential for replication" (specification, p. 21, l. 11).

gene expression region, the vector expressing an essential AAV protein;

(b) preparing a cell capable of producing AAV by introducing a recombinant AAV vector into a host cell;

(c) infecting said cell with said recombinant virus in an amount effective to stimulate the production of recombinant AAV virions, and

(d) culturing said infected cell to obtain the recombinant AAV virions.[3]

49. A recombinant AAV virion prepared by the process of claim 30, which includes a vector construct comprising a nucleic acid sequence encoding a full length CFTR protein, the vector capable of expressing the entire CFTR protein.

The references relied on by the examiner are:

Post et al. (Post), "A Generalized Technique for Deletion of Specific Genes in Large Genome: α Gene 22 of Herpes simplex Virus 1 Is Not Essential for Growth," Cell, Vol. 25, pp. 227-32 (1981)

Haj-Ahmad et al. (Haj-Ahmad), "Development of a Helper-Independent Human Adenovirus Vector and Its Use in the Transfer of the Herpes Simplex Virus Thymidine Kinase Gene," Journal of Virology, Vol. 57, No. 1, pp. 267-74 (1986)

Drumm et al. (Drumm), "Correction of the Cystic Fibrosis Defect In Vitro by Retrovirus-Mediated Gene Transfer," Cell, Vol. 62, pp. 1227-233 (1990)

Muzyczka, "Use of Adeno-Associated Virus as a General Transduction Vector for Mammalian Cells," Current Topics in Microbiology and Immunology, Vol. 158, pp. 97-129 (1992)

[3] A virion is the complete viral particle, which may or may not have an envelope, which serves to transfer the viral nucleic acid from one cell to another (see MOLECULAR BIOLOGY AND TECHNOLOGY: A Comprehensive Desk Reference, p. 953 (Robert A. Meyers, ed.,VCH Publishers, Inc., New York, 1995) (copy attached).

ISSUES

Claim 49 stands rejected under 35 U.S.C. § 103 as unpatentable over Muzyczka and Drumm. Claims 1-6, 8-26, 29-34, 36-40 and 42-46 stand rejected under 35 U.S.C. § 103 as unpatentable over Haj-Ahmad and Muzyczka. Claims 7 and 35 stand rejected under 35 U.S.C. § 103 as unpatentable over Haj-Ahmad and Muzyczka as applied to claims 1-6, 8-26, 29-34, 36-40 and 42-46 and further in view of Post. Claims 27, 28 and 41 stand rejected under 35 U.S.C. § 103 as unpatentable over Haj-Ahmad and Muzyczka as applied to claims 1-6, 8-26, 29-34, 36-40 and 42-46 and further in view of Drumm. We sustain the rejection of claim 49 and reverse the rejections of claims 1-46 under § 103.

In reaching our decision in this appeal, we have given careful consideration to the appellants' specification and claims and to the respective positions articulated by the appellants and the examiner. We make reference to the examiner's answer (Paper No. 13, mailed June 27, 1995) for the examiner's reasoning in support of the rejections and to appellants' brief (Paper No. 12, filed March 28, 1995) for the appellants' arguments thereagainst.

According to appellants, "[t]he claims stand or fall together within each of the four individual rejections" (brief, p. 4). We therefore limit our discussion to claims 1, 7, 27 and 49. 37 CFR § 1.192(c)(5)(1994).

BACKGROUND

The claimed invention relates to recombinant adeno-associated virus (AAV), its production and use, e.g., for transferring exogenous genes into human cell lines and in gene therapy regimens (specification, p. 2, ll. 9-19). According to the specification,

> [i]n recombinant AAV, key viral genes [i.e., "essential" genes] (such as cap, lip and rep) are replaced by the exogenous gene of interest. Methods for producing recombinant AAV therefore rely on co-transfecting the AAV vector carrying the gene of interest, together with a helper AAV plasmid that expresses all of the essential AAV genes, into adenovirus- or herpes-infected [host] cells which supply the helper functions necessary for AAV replication and the production of new viral particles.
>
> The use of cells infected with helper adenovirus or herpes virus does not create a problem, it is the transfection of the essential AAV genes which is the limiting step for the production of high titre AAV virus. [Page 5, ll. 3-15.]

The AAV production methods described in the specification do not require a transfection step (specification, p. 2, ll. 15-16; p. 6, ll. 10-12; p. 32, ll. 2-6). Rather, essential AAV genes are introduced into a recombinant viral vector, i.e., adenovirus or herpes virus, e.g., by inserting the AAV gene(s) into the viral genome or, more generally, by deleting a viral gene and introducing the essential AAV gene(s) in its place (specification, para. bridging pp. 8-9). The recombinant virus expressing the essential gene(s) then supplies the(se) gene(s) to the host cells by infection (specification, p. 32, ll. 20-28).

OPINION

1. Rejection of claim 49 under § 103 over Muzyczka and Drumm

Muzyczka reviews the biology of adeno-associated virus (AAV) and its use as a general transduction vector for mammalian cells. According to Muzyczka, AAV is a human virus which, except under special circumstances, requires coinfection with a helper virus, e.g., a herpes or adeno virus, to replicate. In the absence of a helper virus, AAV establishes a latent infection in which its chromosome is integrated into the host chromosome. (§ 1, pp. 97-98; Fig. 1). The first use of AAV as a viral transduction vector comprised replacing the AAV capsid gene with nonviral DNA (i.e., bacterial neomycin resistance gene under control of the SV40 early promoter) to produce a recombinant plasmid which was then transfected into human cells that had been infected with adenovirus. The cells were cotransfected with a second plasmid, itself defective for packaging but containing a wild type capsid gene, to supply the missing capsid protein. The resulting virus stock contained both adenovirus and AAV recombinant virus. Adenovirus was inactivated by heat or removed by density gradient centrifugation. (§ 4, pp. 110-112; Fig. 6). According to Muzyczka, rep^- AAV vectors are attractive candidates for human gene therapy because (1) the cloning capacity of 5 kb can accommodate a variety of cDNAs, (2) the transduction frequency in human cells is high, (3) no disease has been associated with AAV in either human or animal populations, (4) AAV proviruses appear to be stable, and (5) in the absence of the rep

gene, the AAV terminal repeats appear to be transcriptionally neutral. However, (a) the procedures for growing recombinant virus stocks are awkward, although not technically insurmountable, (b) the mechanism for AAV integration is not known, and (c) relatively little is known about the effect of AAV infection or integration on primary cells. (§ 6, pp. 122-123).

Drumm used a retrovirus vector to transduce CFTR cDNA into a cell line that stably expresses the chloride transport abnormalities characteristic of cystic fibrosis (CF) and found that expression of the normal CFTR gene conferred cAMP-dependent Cl channel regulation on CF epithelial cells (summary, p. 1227).

According to the examiner,

> claim 49 differs from Muzyczka's recombinant AAV virions solely by specifying the inclusion of DNA encoding CFTR. ... It would have been obvious to insert Drumm's CFTR cDNA into Muzyczka's AAV vector in order to take advantage of the desirable characteristics of AAV vectors in providing gene therapy for cystic fibrosis. [Answer, p. 4, para. 1.]

Appellants argue there is no motivation to combine Muzyczka and Drumm because Muzyczka fails to provide a reasonable expectation of success of producing recombinant AAV viral stocks given the problems of contamination, spontaneous deletion of heterologous sequences, low viral titers and instability described by Muzyczka at p. 115 et seq. (brief, pp. 7-10).

"Obviousness does not require absolute predictability of success.... For obviousness under § 103, all that is required is a reasonable expectation of success."

In re O'Farrell, 853 F.2d 894, 903-04, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988). Here, while Muzyczka characterizes the procedures for growing recombinant AAV virus stocks as "awkward," Muzyczka also states "this does not appear to an insurmountable technical problem" (p. 122, last para.). Indeed, Muzyczka identifies several methods for addressing problems in growing AAV stocks. For example, heat inactivation, CsCl density centrifugation and/or anti-adenovirus neutralizing antibody can be used to remove adenovirus contamination (Fig. 6, p. 116, last para.). Use of a complementing plasmid having no homologous sequences between the recombinant genome and the complementing plasmid is disclosed as producing recombinant virus titers of 10^4 to 10^5 with no detectable wild type contamination (citation omitted) (p. 116, first full para.). Instability seen in plasmids propagated in standard RecA prokaryotic hosts, e.g., can be solved by propagating AAV vectors in host such as JC8111 (p. 117, para. 1). The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation "must not be unduly extensive." Atlas Powder Co. v. E.I. DuPont De Nemours & Co., 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984). In this case, Muzyczka provides one of ordinary skill in the art with specific guidance in constructing and growing an AAV vector containing a nonviral DNA sequence. Therefore, this argument is not persuasive.

The rejection of claim 49 under § 103 over Muzyczka and Drumm is sustained.

2. Rejection of claims 1-6, 8-26, 29-34, 36-40 and 42-46 under § 103 over Haj-Ahmad and Muzyczka

As discussed above, Muzyczka discloses replacing an essential gene[s] in the AAV genome with a foreign DNA to form a recombinant AAV vector for introducing the foreign DNA into the DNA of a host cell. This recombinant AAV vector and a plasmid carrying the deleted essential AAV gene are cotransfected into a host cell infected with an adeno or herpes virus.

Haj-Ahmad discloses adenovirus vectors wherein "nonessential" E1 and/or E3 regions of the virus have been replaced by a foreign DNA (N.B. E3 is nonessential for adenovirus replication in cultured cells, while E1 is nonessential when the virus is propagated in 293 cells which constitutively express the E1 gene products) (abstract).

According to the examiner, "it would have been obvious to insert the essential AAV genes into an adenovirus as a convenient way of introducing both an adenovirus and the essential AAV genes together into host cells for the production of AAV vectors" (answer, p. 5, para. 1).

We have no doubt that the prior art could be modified in the manner proposed by the examiner, however the fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification. In re Gordon, 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed. Cir. 1984). Here, we agree with appellants that the examiner has not established a prima

facie case of obviousness because the prior art does not disclose or suggest that essential AAV gene(s) should be incorporated into an adenoviral or herpesviral genome (brief, p. 10). The examiner has not pointed out, and we do not find, where Haj-Ahmad discloses or suggests that the essential AAV gene, found only in the second, nonreplicating plasmid of Muzyczka, should be inserted into the helper virus of Muzyczka in order to compensate for defects in the AAV vector of Muzyczka.

The adenovirus vector of Haj-Ahmad delivers its inserted foreign DNA into the genome of the host cell. The examiner has not explained why one of ordinary skill in the art would create an adenovirus vector with a foreign DNA which would not be stably integrated into the genome of the host cell. The examiner has not explained why one of ordinary skill in the art would combine the helper-independent viral vector system of Haj-Ahmad with the helper-dependent viral vector system of Muzyczka. In our judgment, the only reason or suggestion to combine the references in the manner proposed by the examiner comes from appellants' specification. Thus, we find the examiner has not carried his burden of establishing a prima facie case of obviousness and has relied on impermissible hindsight in making his determination of obviousness. In re Fritch, 972 F.2d 1260, 1266, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992) ("It is impermissible to engage in hindsight reconstruction of the claimed invention, using the applicant's structure as a template and selecting elements from references to fill the gaps.).

Accordingly, the rejection of claims 1-6, 8-26, 29-34, 36-40 and 42-46 under 35 U.S.C. § 103 as being unpatentable over Haj-Ahmad and Muzyczka is reversed.

3. Rejection of claims 7 and 35 under § 103 over Haj-Ahmad, Muzyczka and Post

4. Rejection of claims 27, 28 and 41 under § 103 over Haj-Ahmad, Muzyczka and Drumm

The examiner relies on Post to suggest using a herpes virus as an alternative to adenovirus as a vector for delivering genes; and, on Drumm for disclosing CFTR cDNA and suggesting its use in treating cystic fibrosis (answer, pp. 6-8). However, the examiner has not pointed out, and we do not find where, Post and/or Drumm disclose or suggest the required limitation of a recombinant adenovirus or herpesvirus vector comprising an essential AAV gene capable of expressing an essential AAV protein.

Accordingly for the reasons stated above, we do not sustain any of the examiner's rejections of claims 1-46 under 35 U.S.C. § 103 as unpatentable over any combination based on Haj-Ahmad and Muzyczka.

OTHER MATTERS

We note that a requirement for a new oath or declaration in compliance with 37 CFR 1.67(a) is still outstanding (see final Office action, p. 2, Paper No. 9, mailed October 3, 1994). We also note that claims 28-30 and 46 do not recite a recombinant AAV vector lacking an essential AAV gene and an adenovirus vector comprising the essential AAV gene lacking from the AAV vector. The examiner should take a step back and determine whether this raises any issues under the 35 U.S.C. § 112, second paragraph, requirement that the claims "particularly point out" the subject matter which applicants regard as their invention.

CONCLUSION

In summary, the decision of the examiner (1) to reject claim 49 under 35 U.S.C. § 103 as unpatentable over Muzyczka and Drumm is affirmed, (2) to reject claims 1-6, 8-26, 29-34, 36-40 and 42-46 under 35 U.S.C. § 103 as unpatentable over Haj-Ahmad and Muzyczka is reversed, (3) to reject claims 7 and 35 under 35 U.S.C. § 103 as unpatentable over Haj-Ahmad, Muzyczka and Post is reversed and (4) to reject claims 27, 28 and 41 under 35 U.S.C. § 103 as unpatentable over Haj-Ahmad, Muzyczka and Drumm is reversed.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

AFFIRMED-IN-PART

WILLIAM F. SMITH Administrative Patent Judge	)
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